

ASSESSING THE COMPATIBILITY OF PACKED RED BLOOD CELLS WITH
LACTATED RINGER'S SOLUTION

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ABSTRACT

Crystalloid solutions are frequently used to reconstitute packed red cells during rapid infusions during trauma situations and in the perioperative setting. Lactated Ringer's solution is a commonly used crystalloid for intravenous infusion. Calcium contained in lactated Ringer's solution has been reported to possibly cause blood clots because of replacing calcium ions previously chelated in the citrate anticoagulant. Authors of previous studies have cautioned against using lactated Ringer's solution to reconstitute packed red cells because of this calcium. Other studies have shown that there are no adverse effects when lactated Ringer's is used in situations when packed red cells are transfused rapidly. In this study, samples of CPD-preserved red cells were diluted with either lactated Ringer's solution or normal saline. The aliquots were diluted to ratios between 10:1 and 1:10 (packed red cells to crystalloid) and incubated at room temperature and 37 degrees centigrade and then examined for clot formation at intervals up to two hours. Clotting occurred at dilutions of 1:1 and higher, and no clots formed in clinically relevant dilutions between 5:1 and 2:1. Additional units of CPD-preserved blood were diluted to hematocrit values of 35, 45, 55 and 65 percent and were passed through blood tubing to simulate an actual transfusion. Flow rates of packed red cells diluted with lactated Ringer's solution and normal saline were compared. There was no measurable difference in the flow rates.

KEY WORDS: packed red cells, lactated Ringer's, compatibility, coagulation, calcium.

ASSESSING THE COMPATIBILITY OF PACKED RED BLOOD CELLS WITH LACTATED
RINGER'S SOLUTION

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CHAPTER ONE: INTRODUCTION

Background of the Problem

Many intravenous (IV) solutions are available today, and a thorough knowledge of the underlying fluid balance assists the Certified Registered Nurse Anesthetist (CRNA) in understanding why a particular fluid is required. Intravenous fluids are classified as hydrating, balanced, and gastrointestinal solutions (Kuhn, 1991), and each has a place in treating fluid and electrolyte imbalances or potential imbalances within the perioperative setting. Hydrating solutions are composed of water, carbohydrates, and varying amounts of sodium chloride. These solutions are used primarily for simple fluid replacement and hydration and to determine the adequacy of a patient's renal function. Balanced solutions are composed of water, carbohydrates as an energy source, and contain a balance of cations and anions. Balanced solutions are rarely used in the perioperative setting today, because their use may cause electrolyte imbalances. Replacement solutions are usually a combination of water, carbohydrates, and electrolytes that are used to replace concurrent losses from the gastrointestinal (GI) system, which occur commonly during surgery. These solutions are composed of various electrolytes that approximate losses from various GI areas through vomiting, diarrhea, fistulas, and suction. Lactated Ringer's solution is a common multipurpose system that is often used both in a perioperative setting and trauma treatment.

When a patient requires blood component therapy concomitant with IV fluid administration, the CRNA must be fully aware of any contraindication to the concurrent administration of the blood with a particular fluid. The American Association of Blood

Banks (AABB), the American Red Cross, and the Council of Community Blood Centers have jointly prepared a circular with the approval of the Center for Biologics and Research of the Food and Drug Administration (FDA) that provides strict guidelines for the use of human blood and blood components (AABB, 1995). The CRNA must be fully cognizant of the contents of this blood product circular of information.

The Technical Manual of the AABB (1995) has summarized these standards as being:

explicit in stating that medications must not be added to blood or components. If red cells require dilution to reduce their viscosity or if a component needs to be rinsed from the blood bag or tubing, normal saline (0.9% sodium chloride injection, USP) can be used as the product of choice. Red cells prepared with an additive solution ordinarily do not require dilution. Other solutions intended for intravenous use may be added to blood or components or may come into contact with blood in an administration set only if they have been approved by the Food and Drug Administration (FDA) or if there documentation to show that their addition to blood is safe and efficacious. Calcium-free, isotonic electrolyte solutions that meet the above requirements may also be used.

Solutions not approved for addition to blood components or for simultaneous administration via the same intravenous line include lactated Ringer's solution, 5% dextrose in water, and hypotonic sodium chloride solutions. Dextrose solution may cause red cells to clump in the tubing and, more important, to swell and hemolyze as dextrose and associated water diffuse

from the medium into the cells. Lactated Ringer's solution contains enough ionized calcium (3mEq/L) to overcome the chelating agents in anticoagulant-preservative or additive solutions and allow small clots to develop. (p.451-2)

Healthcare providers including CRNAs are limited in which fluids may be mixed with blood products in the perioperative setting. There have been complications reported with massive infusions of saline-containing solutions (Brown, Kim, Weeks, & Parkin, 1978). These clinical complications include dilutional acidosis, edema, and hypokalemia. Therefore, the CRNA must be judicious in mixing saline with blood products, as when attempting to increase the flow rate of a blood transfusion.

CRNAs frequently apply the knowledge that is developed within the basic sciences to guide their safe practice of administration of anesthetics to patients in many varied settings. It is only through the continuing development of the knowledge base that is required for practice that the profession continues to grow. The CRNA as a nursing professional is dedicated through a spirit of scientific curiosity and a continuous desire for new and better ways of doing procedures within practice settings.

A good example of the use of previously-developed scientific knowledge by CRNAs is their frequent use of intravenous (IV) therapy in the perioperative setting. Patients may require the administration of IV fluids because of (a) an inability to orally ingest adequate amounts of fluids, electrolytes, vitamins, or calories; (b) a fluid or electrolyte imbalance; or (c) a significant loss of blood volume (Kuhn, 1991). Intravenous therapy involves infusing many different types of fluids directly into the circulatory system. The fluids that may be

administered include dextrose solutions, electrolyte solutions, nutritional preparations, plasma expanders, blood and blood components. Obviously, the potential exists for iatrogenic incompatibilities between the many different solutions that may be administered, particularly when combined with the full spectrum of blood component therapy.

Rationale and Significance of the Problem

Since CRNAs routinely use lactated Ringer's solution as a replacement solution during surgery, it would seem obvious that they would want to mix a potential infusion of a blood product with that solution that is already infusing. However, as stated above, in many instances this is not presently permitted by the American Association of Blood Banks and the Food and Drug Administration (FDA).

The compatibility of lactated Ringer's and anticoagulated blood products have received only sporadic attention throughout the years. The most recent edition of the AABB Technical Manual (AABB, 1995) contains only three references pertaining to the mixing of lactated Ringer's and anticoagulated blood products. Ryden and Oberman (1975) conducted the first of these studies using citrate-phosphate-dextrose (CPD) anticoagulated whole blood. Other researchers partially replicated this work in several different years. In 1980, Dickson and Gregory used a different technique to observe coagulation. In 1989, Strautz, Nelson and Schulman used packed red cells preserved with adenine-saline-dextrose solution (ADSOL). In 1991, Cull, Lally and Murphy used packed red cells preserved with citrate-phosphate-dextrose-adenosine (CPDA). All of these investigators observed fibrin clots in vitro at a blood:solution ratio of 10:1 or lower. The three studies presented a common reason for the presence of the clots: the calcium

present in the lactated Ringer's solution exceeds the anticoagulant properties of the citrate found in the anticoagulated and preserved blood products (Cull, Lally & Murphy, 1991; Dickson & Gregory, 1980; Ryden & Oberman, 1975; Strautz, Nelson & Schulman, 1989).

It is intriguing to note, however, that in contrast to these in vitro studies, there are studies that indicate that the in vivo use of lactated Ringer's solution mixed with anticoagulated blood products in trauma and perioperative settings have caused no apparent harm to patients (Schwab, Shayne & Turner, 1986; Shackford, Virgilio, & Peters, 1980). Many practicing anesthesia providers admit that lactated Ringer's solution was, in the past, mixed with blood products in their practice, particularly in the reconstitution of viscous red blood cells. This often occurred in settings where high flow rates were desired, often in support of trauma patients. Apparently, this practice was curtailed with the publication of the prohibition against lactated Ringer's use with blood products by the FDA in Circular of Information (AABB, 1995). A review of the literature will examine these in vivo and in vitro studies in depth.

Statement of the Problem

This research was a replication of the Ryden & Oberman (1975) study using the methodology of Cull, Lally, & Murphy (1991) in order to validate or refute their recommendation that lactated Ringer's solution should never be administered concurrently with blood or that it may be harmful when used to start transfusions as "it rapidly produced clots when mixed with CPD blood" (Ryden & Oberman. P. 250). This research modified the original study by including the most recent anticoagulants and preservatives.

It used packed red cells rather than whole blood since presently whole blood is rarely indicated in normal transfusion practices (AABB, 1995). Additionally, this research included the mixing of anticoagulated packed red cells with lactated Ringer's solution and simulated an infusion at high IV flow rates.

Research Questions

1. What is the effect of mixing lactated Ringer's solution with anticoagulated packed red blood cells?
2. Do IV flow rates have a measurable influence on clotting?
3. Is there an upper limit of a ratio of blood to lactated Ringer's solution at room and body temperature, where clots are no longer observed?

Conceptual/Theoretical Framework

Since this research was of a quantitative, basic science nature, the nursing theory that would guide the research needed to direct nursing education in the biological sciences. Akinsanya's (1987) model of bionursing is one of these. "It is a framework for the delivery of biological science knowledge to students of nursing (Casey, 1996, p. 1065). The bionursing model seeks to remove nursing's dependence on the biomedical model in science education. The purpose of Akinsanya's (1987) model was to develop a link between nursing and the life sciences separate from the traditional link through medicine. Two major underlying assumptions in this theory are that nurses require a knowledge of the biological sciences for practice and that nursing is a task based profession. Akinsayna further proposed that the use of the biological sciences in the teaching and practice of nursing should be termed bionursing.

The main concepts in the model are bionursing, 4-level task performance, and care (Akinsanya, 1987). Basically, the four levels of task performance guide a nursing student's progress during their education as their professional responsibilities and scope of practice develop. The concept of care consists of three elements. The first involves the routine tasks involving skills and techniques rooted in the life sciences. The second involves "decision-making within the limits of professional responsibility, which is crucially dependent on a full understanding of which lies mainly in the behavioral and social sciences" (Casey, 1996, p. 1066). The third element of care is comprised of interpersonal relationships, the understanding of which lies in the social and behavioral sciences.

Bionursing, then, provides a theoretical framework that would guide a researcher through a scientific quantitative study. It proposed a distinct type of nursing knowledge termed bionursing in which scientific knowledge impacts bionursing, and it provides the rationale for the tasks of nursing care and for informed professional decision making. The theory allows a CRNA to question many aspects of the professional practice of anesthesia and to continually seek to improve the scientific knowledge base.

Nursing and Military Relevance

There would be numerous benefits to the military and nursing communities if lactated Ringer's solution could be routinely mixed with blood during transfusions. There would be a cost savings in terms of the amount and types of crystalloid solutions needed to be on hand to be mixed with blood. There would also be a savings in terms of the equipment required to transfuse blood in terms of a reduced amount of specialized blood tubing.

Primarily patients would benefit by receiving blood products at much higher flow rates. This would be particularly important during the management of patients who have hypovolemic shock. Patients would also benefit directly from a reduced saline load and a concomitant reduction in complications due to the sodium volume. In 1978, Brown, Kim, Weeks & Parkin noted that when physiologic saline solution is used to reconstitute blood, and is given in massive amounts, numerous post transfusion clinical problems may result including dilutional acidosis, edema, hypernatremia and kaliuresis. Since lactated Ringer's solution mimics the concentrations of electrolytes that are lost from the GI tract, it's use in quantities sufficient for volume replacement is clearly better than use of physiologic saline. As noted previously, there are several documented cases where use of lactated Ringer's solution as a volume replacement combined with blood products produced no clinically observable adverse effects (Shackford, Virgilio, & Peters, 1980).

Berger, Monaghan and Hann (1987) proposed a new and novel method of use of frozen intravenous crystalloid solutions as the refrigerant for shipping blood. They noted that fluid and volume replacement are essential for the treatment of hemorrhagic shock after trauma. They also noted that a combination of packed red cells and crystalloid was the most effective form of treatment. "Providing this combination in battlefield or civilian disaster settings requires shipment of the PRBCs under refrigeration" (p.406). Generally, the military has used wet ice to maintain the blood within the temperature range specified by the Code of Federal Regulations in the Standards of the AABB(1995). The results of the study suggested that cooled intravenous crystalloid solutions could be used as refrigerants for PRBCs during shipment. The military would realize a cost savings in at

least two ways—a space savings from not having to use the wet ice; and a savings in cost from not having to purchase additional physiologic saline for PRBC reconstitution or the ice for transportation. Frozen lactated Ringer’s solution could be used in place of both the saline and wet ice. Military echelons of providing medical care rely heavily on the use of crystalloid-colloid solutions (Monaghan, 1983).

Definitions

Certified Registered Nurse Anesthetist:

Denotes a registered nurse (RN) who has received advanced training in anesthesia care and is legally qualified to administer anesthesia. In order to qualify, an RN must graduate from an accredited program, pass the national board exam, and recertify every two years. The terms CRNA and nurse anesthetist may be used interchangeably.

Whole Blood:

Contains the red blood cell and plasma components of donor blood. Most of the platelets and white cells may have been rendered non-therapeutic during the storage process. Whole blood provides red blood cells to carry oxygen to tissues. It also is a blood volume expander and a source of proteins with oncotic and certain nonlabile coagulation properties. Whole blood was once indicated only for those patients who have a symptomatic deficit in oxygen-carrying capacity combined with hypovolemia of sufficient degree to be associated with shock. It is rarely used today.

Red Blood Cells:

Prepared by centrifugal or gravitational separation of the red cells from blood plasma. This blood component increases the oxygen-carrying capacity of the blood by increasing

the red blood cell mass. This component is the component of choice for patients with a symptomatic deficit of oxygen-carrying capacity. It is usually anticoagulated with CPD and preserved with an adenine-saline solution to increase its storage life.

CPD:

Citrate-phosphate-dextrose. The sodium citrate-containing blood anticoagulant. This is added to blood as it is donated to inactivate the clotting cascade by chelating calcium.

ADSOL:

Adenine-saline-dextrose solution. This solution is an adenine-containing solution added to packed red cells to preserve them by promoting anaerobic glycolysis and to improve flow characteristics—i.e. to decrease viscosity.

Coagulation:

The process of clot formation in response to an internal or external stimulus. Donated blood must be anticoagulated to inactivate the natural coagulation process.

Plasma:

Consists of the anticoagulated clear portion of blood that is separated by centrifugation or sedimentation from whole blood. All stable coagulation factors are present.

Assumptions and Limitations

Since this study was conducted entirely in vitro, it may only be used to merely predict what may happen in vivo. Further, more comprehensive studies may need to be done in order to prove safety and validity. Additionally, all units of stored blood used for

experimentation were within three days of expiration. The collected data may have been different if fresher units of blood were used in the experiments.

Summary

This chapter has provided the background of the problem, the rationale and significance of the problem, a statement of the problem, the conceptual framework, the nursing and military relevance of the research, and the assumptions and limitations of the study. Clearly, the compatibility of lactated Ringer's solution with anticoagulated blood products has received only sporadic attention over the years. This limited research has guided and changed the practice of many anesthesia providers. The research was of enough significance that its recommendations were included in federal regulations. The replication of this important work should help to validate or refute the results of that work. The types of preservatives and anticoagulants have changed since Ryden and Oberman conducted their study. Additionally, whole blood is generally no longer transfused routinely. Therefore, a replication of their work is necessary to apply their recommendations to the current state of blood transfusion practice.

CHAPTER TWO: REVIEW OF THE LITERATURE

This chapter will first review the literature that examines the compatibility of lactated Ringer's solution with anticoagulated blood products. In these studies, the investigators evaluated the compatibility of many crystalloids—not just lactated Ringer's solution with anticoagulated blood products. It is important to note that the authors of these studies conducted all of their research in vitro. A review of those scientific reports using lactated Ringer's solution in an in vivo clinical setting where lactated Ringer's solution was mixed with blood products was also explored.

One of the first things noted while conducting the literature review was that there are very few studies relating specifically to the mixing of blood products utilizing lactated Ringer's solution. It was not until 1975, when Ryden and Oberman conducted their study, that lactated Ringer's solution was evaluated for blood component compatibility. That work has only been partially replicated four times—twice using different research methodologies and two additional times using the newer anticoagulants and preservatives. Apparently, there has been little interest in replication of this work since it was incorporated into the standards of the AABB (1995).

There was also been comparatively little published research conducted in the actual use of lactated Ringer's solution in a clinical setting. Those that have been conducted have focused on lactated Ringer's use as a volume replacement in addition to red cell replacement (Schwab, Shayne, & Peters, 1986; Shackford, Virgilio, & Peters, 1980).

The primary focus of these studies was volume replacement in emergency or trauma situations, as well as volume replacement during surgeries. The alleged incompatibility of

lactated Ringer's solution with blood and the prohibition of mixing it with anticoagulated blood as stated in the AABB (1995) standards, has resulted in little interest in pursuing further research in this area.

In 1975, Ryden and Oberman evaluated the compatibility of stored whole blood with various intravenous solutions. While other researchers had previously evaluated various dextrose-containing solutions (Wilson, 1950; Buschle & Saklad, 1953), Ryden and Oberman (1975) performed the first evaluation of mixing lactated Ringer's solution with bank blood anticoagulated with CPD.

In their experiments, Ryden and Oberman (1975) mixed anticoagulated blood with lactated Ringer's solution, 5 percent aqueous dextrose, 5 percent dextrose in 0.225 percent saline, 5 percent dextrose in 0.9 percent saline, and 0.9 percent saline solution in varying concentrations and incubated them at room temperature and 37 degrees centigrade. They found clots in the blood-lactated Ringer's mixture after five minutes at a citrate:calcium molar ratio of 4:1 or lower. Ryden and Oberman also noted immediate clumping in the aqueous dextrose-blood mixture with gross hemolysis, hemolysis of blood mixed with 5 percent dextrose and 0.225 percent saline, and no hemolysis with blood mixed with 5 percent dextrose in 0.9 percent saline or blood mixed with 0.9 percent saline. They also simulated starting blood transfusions through IV tubing already filled with lactated Ringer's solution and 5 percent dextrose in 0.225 percent saline. They then observed the clearance of the solution from the IV tubing. They observed that the residual crystalloid solutions remain in the tubing for up to 30 minutes after initiating a transfusion.

Based on these observations, Ryden and Oberman (1975) recommended that lactated Ringer's solution and 5 percent dextrose in 0.225 percent saline should not be administered concurrently with blood. They also noted that lactated Ringer's solution may be harmful when used to start transfusions, as it rapidly produced clots when mixed with CPD bank blood. They also reported that the "danger of using lactated Ringer's is assumed to be small, since no adverse results have been reported in years of clinical use" (p.254). This assumption may be faulty, since they showed that crystalloid remains in the IV tubing for a period of time after starting a transfusion.

The Ryden and Oberman (1975) study results are undoubtedly correct as an in vitro evaluation of the compatibility. However, as they freely admit, there have been no adverse reports from the use of lactated Ringer's with blood in clinical settings. Their work needed to be replicated for several reasons. First, they performed their experiments using whole blood, which is rarely used in present day clinical settings. Whole blood contains much more calcium than packed red cells, and would therefore be more likely to be effected by the calcium contained in lactated Ringer's solution. Additionally, their work didn't include transfusion simulations at high flow rates, as would be likely in emergency or trauma situations. Finally they didn't determine a blood/Ringer's concentration at which clotting does not occur.

Dickson and Gregory (1980) replicated a portion of Ryden and Oberman's work using a different methodology. They mixed whole blood and lactated Ringer's, along with solutions that did not contain calcium and let them stand at room temperature. The ratio of blood:Ringer's was approximately 1:10. It was not surprising that the solution

produced clots, since the ratio was very low. They did verify the presence of clots with electron microscopy, while Ryden and Oberman (1975) used visual techniques to determine the presence of clots.

Dickson and Gregory (1980) also performed simulated transfusion with blood and lactated Ringer's solution. However, they modified the Ryden and Oberman (1975) study procedures by including experiments that began with a blood administration set filled at the outset with blood or lactated Ringer's solution. This had the effect of comparing various concentrations of blood versus lactated Ringer's solution in the administration sets at the beginning of the experiments. They observed clots in the tubing at flow rates of 60 drops per minute.

Dickson and Gregory (1980) also recommended that whole blood anticoagulated with CPD not be mixed with solutions containing calcium and proposed the use of a formulation of lactated Ringer's solutions that did not contain calcium. Like Ryden and Oberman (1975), these researchers could not offer any evidence that the clinical use of lactated Ringer's solution caused no apparent harm to patients. They only assumed that the clots would be produced and would have to be filtered out in the lungs. Their results corroborated the Ryden and Oberman study. Their lack of changing any significant parameters, i.e. flow rates, limits the use of this study to the verification of Ryden and Oberman's findings.

In 1988, King, Patten, and Bee identified a threshold value for ionized calcium (0.23 mM/L) below which the probability of clot formation is less than 0.01. To do this, they mixed packed red cells with lactated Ringer's solution and measured the resulting

solutions pH, ionized calcium, and total calcium. Additionally, they mixed units of packed red cells with lactated Ringer's solution and normal saline and filtered the units through blood tubing and weighed the filters afterwards to assess for a weight difference that might be due to clot formation. They concluded that the threshold concentration is not reached unless the packed red cell to lactated Ringer's volume ratio is 2:1 or greater. This ratio is not exceeded when as much as 100 ml of lactated Ringer's solution is added to a unit of packed red cells. They did not advocate the routine use of lactated Ringer's solution as a diluent for packed red cells, but they concluded that it may be done.

In 1991, Cull, Lally, and Murphy also mixed packed red cells with lactated Ringer's solution and studied the flow rates of units of packed red cells diluted with lactated Ringer's solution and normal saline. They diluted samples of CPD-preserved packed red cells with either lactated Ringer's solution or normal saline in ratios between 5:1 to 1:20 (packed red cells to crystalloid) and incubated them at room temperature and 37 degrees centigrade. They examined these mixtures at various time intervals for two hours and observed clotting at ratios of 1:1 and beyond. They discovered that no clot formation occurred at clinically relevant dilution between 5:1 and 2:1.

Cull et al. (1991) also diluted units of packed red cells to hematocrit values of 35, 45, 55, and 65 percent with lactated Ringer's solution and normal saline and compared the flow rates when the solutions were passed through blood tubing filters. They found no difference in the flow rates. They concluded that lactated Ringer's solution could be safely used as a packed red cell diluent in patients requiring rapid blood transfusions.

Strautz, Nelson, Meyer, and Schulman (1989) examined the compatibility of ADSOL-stored red cells with various intravenous solutions. They partially replicated Ryden and Oberman's 1975 work using 5 percent dextrose in water and lactated Ringer's solution. The major difference was the use of the packed red cells in place of the previous study's use of whole blood. Since there is much less plasma in packed red cells preserved with ADSOL, Strautz et al. (1989) stated that "clinicians may feel the mixing of RL with AS 1-RBCs is a safe practice because of the reduced plasma volume. No studies have been published that address whether or not it is safe to add RL or 5% dextrose solutions to AS 1-RBCs" (p.162).

Strautz et al. (1989) observed clots at different ratios of blood to lactated Ringer's from that which was previously reported by Ryden and Oberman(1975). This should have been an expected result, since with a reduced amount of plasma in the mixture, there would be less calcium available to initiate the clotting cascade. Based on their results, these researchers recommended that ADSOL-stored red blood cells not be mixed with Lactated Ringer's solution. They did not perform any simulated transfusion experiments. Therefore, there is no way to know if the ADSOL-stored red cells would clot in blood administration sets. Strautz et al. (1989) did offer documentation of the use of lactated Ringer's solution in clinical studies and offered a critique of that work.

Shackford, Virgilio, and Peters (1981) studied the use of packed red cells reconstituted with lactated Ringer's solution versus whole blood in patients undergoing major aortic reconstruction surgeries. They divided the patients in to groups based on the use of whole blood or packed red cells. The patients' cardiac index, pulmonary capillary

wedge pressure, colloid osmotic pressure, platelets, prothrombin time, partial thromboplastin time, and fibrinogen levels were measured before, during and for three days postoperatively. The researchers found no significant differences between the groups other than an expected greater decrease in colloid osmotic pressure in the group receiving the packed red cells. The study supported the safety of blood replacement with packed red cells and lactated Ringer's solution to provide effective volume replacement without producing coagulopathy. The Shackford et al. (1981) study findings support the notion that there are no ill effects from mixing red cells with lactated Ringer's solution in vivo.

Strautz et al. (1989) observed that in the Shackford et al. study that:

the authors did observe a decrease in the measurement of circulating platelets and and fibrinogen immediately following the use of RBCs that were reconstituted with RL. This decrease was greater than that noted after the use of whole blood. This finding might have been caused, at least in part, by the activation of the clotting in units of RBCs to which RL was added. This in turn might have initiated a subclinical episode of disseminated intravascular coagulation (DIC) in the patients to some degree (p. 164).

What Strautz et al. (1989) stated was indeed true. However, the same changes in patient parameters were noted in the whole blood group. Most importantly, however, was that the measured patient parameters in both groups returned to normal by the first day after the operation.

Schwab, Shayne, and Turner (1986) conducted an evaluation of the immediate trauma resuscitation with uncrossmatched type O packed red cells that they reconstituted with

either normal saline or lactated Ringer's solution. All of the patients that they studied met specified criteria for requiring "massive transfusion". Although many of the patients in the study died, no death was directly attributed to a transfusion reaction. They concluded that uncrossmatched type O blood reconstituted with normal saline or lactated Ringer's solution was safe to use in emergency trauma situations.

Strautz et al. (1989) criticized the Schwab et al. (1986) study by stating that "50% of the patients either died within minutes of arrival at the trauma center or developed complications such as acute respiratory distress syndrome (ARDS) and disseminated intravascular coagulation (DIC)" (Strautz, et al., 1989, p.164). While this was indeed true, they offer no evidence as why the deaths would have been related to the blood transfusions rather than to the severity of their condition as a result of massive trauma.

Thus the literature review has revealed that in a laboratory in vitro setting, when any form of anticoagulated blood component is mixed with lactated Ringer's solution clots may form. However, literature also indicates that lactated Ringer's use in an vivo clinical settings has produced no observed or measurable ill effects in patients. Clearly, then, this topic begs more research to fully assess the issues of biocompatibility. Lactated Ringer's solution is an excellent intravenous solution for use in various clinical settings. If the benefits of its' use outweigh the potential problems caused when it is mixed with anticoagulated blood products, then its' use should not necessarily be restricted.

CHAPTER THREE: METHODOLOGY

Introduction

This study was a partial replication of the earlier work performed by Ryden and Oberman in 1975 using the materials and methods of the 1991 replication of that study by Cull, Lally and Murphy. As in the Cull et al. study, this study used packed red cells preserved with citrate phosphate dextrose adenosine (CPDA) rather than whole blood. Experiments were conducted to determine at what ratios of packed red cells would clot when mixed with lactated Ringer's solution. Additional experiments measured the flow rates of units of packed red cells when diluted to various hematocrits and passed through standard blood tubing.

Research Design and Procedures

Data was obtained through laboratory experimentation using in-date packed red blood cells collected from volunteer donors and preserved with CPDA1. Crystalloid solutions, blood administration tubing, intravenous catheters and other supplies were obtained from commercial sources. The materials were obtained in sufficient quantities to performed adequate numbers of measurements for statistical significance. The same brands of crystalloid solution, blood tubing, and intravenous catheters were used to account for any possible variability of equipment between manufacturers. Laboratory equipment used for experimentation was a part of the U.S. Navy's equipment calibration program and had current certification.

Measurement

Four units of in date packed red cells anticoagulated with CPDA1 were used in the first part of the study. Four samples of blood, one from each unit of packed red cells were diluted to volume ratios of packed red cells to crystalloid of 10:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:5, and 1:10 with normal saline and lactated Ringer's solution. The samples were mixed in glass centrifuge tubes at 37 degrees centigrade in a water bath and at room temperature to determine if clotting and temperature are related. The samples were examined visually at six time periods: 5, 10, 20, 40, 60 and 120 minutes. Clots were determined by the observance of fibrin strands on orangewood sticks. The samples were stirred with the orangewood sticks before each observation for clots to ensure uniform mixing within the sample and to break up any agglutinations not due to clot formation. Twenty-four additional units of packed red blood cells were diluted with either normal saline or lactated Ringer's solution to hematocrits of 35, 45, 55 and 65 percent. These were used to determine if blood clots not detectable by gross examination could effect flow rates through actual blood tubing filters. Crystalloid solution was injected directly into the units of packed red cells and manually mixed for five minutes. Flow rates were then measured using gravity flow at a fixed height of six feet through standard blood tubing and a 16 gauge IV catheter. The time it took each unit to empty was recorded to the nearest second. The flow rates were then determined by measuring the weight of the full bag of blood versus the empty bag. The density of the diluted packed red cells was assumed to be 1 gram per cubic centimeter.

Instrumentation

The presence of clots were graded according to the parameters specified by Strautz et al. (1989) in their examination of ADSOL-stored red blood cells (Appendix A). The procedures of the experiments and the methods of grading the clot formation were validated by a clinical laboratory specialist in the Blood Bank Department of the National Naval Medical Center, Bethesda, Maryland. The clinical laboratory specialist also verified that the data collector was sufficiently trained to perform the experiments.

Plan for Data Analysis

The data was tabulated, reviewed and verified by a clinical laboratory specialist to assure accuracy. Only four experiments were conducted in the first part of the study and twenty four in the second part, due to the limited supply of in date packed red blood cells. The results of the experiments were very similar and therefore limited the statistical analysis.

Summary

This work partially replicated a previous study to determine it's validity and applicability to present-day transfusion practices. It reinforces the results of the previous study as being valid.

CHAPTER IV: ANALYSIS

Presentation, Analysis, and Interpretation of Data

When packed red blood cells were mixed with normal saline solution, no clots were observed at any of the volume ratio dilutions or temperatures. Clots did occur in volume ratio dilutions of packed red cells to lactated Ringer's solution between 1:1 and 1:10. Clots appeared first in volume ratio dilutions of 1:1, 1:2, and 1:3 when incubated at 37 degrees. When incubated at room temperature (20 degrees centigrade), clots appeared first at volume ratio dilutions of 1:2, 1:3, and 1:5, also appearing first at ten minutes. As in the Cull et al. (1991) study, there was no evidence of clotting in the volume dilutions of 10:1, 5:1, 3:1, or 2:1 at either room temperature or 37 degrees centigrade.

Table 1 illustrates the coagulation profiles of the 216 samples diluted with lactated Ringer's solution or normal saline. All of the samples within each volume dilution and temperature displayed the same propensity to clot. The strengths of clots observed varied from 1+ to 4+.

Packed red cells were also diluted with lactated Ringer's solution and normal saline to hematocrit percentages of 35, 45, 55, and 65 (Table 2). The units were infused utilizing gravity through standard blood tubing sets at wide-open flow rates through a 16 gauge IV catheter. The average flow rates were computed and were found to be the same for units diluted with normal saline or lactated Ringer's solution. The solutions were observed for two hours after passing through the blood filters and examined for clots. No clots were found in any of the samples.

The flow rates of PRBCs and normal saline varied from 40.2 cc/minute at a hematocrit of 65% to 59.1 cc/minute at a hematocrit of 35%. The flow rates observed of PRBCs and lactated Ringer's solution varied from 39.6 cc/minute at a hematocrit of 65% to 58.8 cc/minute at a hematocrit of 35%. No individual flow rate varied by more than two cc/minute from the average flow rate for each individual hematocrit.

Table 1

Clotting Profiles of PRCs Diluted with LR at Room Temperature and 37 degrees

PRC:LR	Time (minutes)											
	5		10		20		40		60		120	
Volume Ratio	RT	37	RT	37	RT	37	RT	37	RT	37	RT	37
10:1	0	0	0	0	0	0	0	0	0	0	0	0
5:1	0	0	0	0	0	0	0	0	0	0	0	0
3:1	0	0	0	0	0	0	0	0	0	0	0	0
2:1	0	0	0	0	0	0	0	0	0	0	0	0
1:1	0	0	0	1+	2+	3+	3+	4+	4+	4+	4+	4+
1:2	0	0	1+	1+	2+	4+	3+	4+	4+	4+	4+	4+
1:3	0	0	1+	1+	2+	4+	3+	4+	4+	4+	4+	4+
1:5	0	0	1+	0	3+	2+	3+	3+	3+	3+	4+	4+
1:10	0	0	0	0	1+	1+	1+	1+	3+	3+	4+	4+

n = 4

Table 2

Average Flow Rates of PRCs Diluted With Normal Saline and Lactated Ringer's Solution

Hematocrit									
Percent	35		45		55		65		
	NS	LR	NS	LR	NS	LR	NS	LR	
Flow Rate in	59.1	58.8	55.9	56.1	41.7	41.3	40.2	39.6	
cc per minute									

n = 3

CHAPTER V: CONCLUSIONS AND RECOMMENDATIONS

The primary purpose of this research was to replicate the earlier work of Ryden and Oberman (1975) using the materials and methodology of Cull, Patten and Bee (1991) to determine if the results of the studies are valid. Research questions that needed to be answered were: What is the effect of mixing lactated Ringer's solution at room and body temperatures where clots are no longer observed?. This research was able to answer those questions. At certain ratios, packed red blood will indeed clot when mixed with lactated Ringer's solution; packed red blood cells do not appear to clot in blood tubing when infused at high flow rates; and there is an upper limit at room and body temperatures where clots are not observed. This research was able to verify that the results of the Cull et al. study appear to be valid.

The review of the literature revealed that relatively little research has been performed on the topic of red cell compatibility with calcium-containing solutions since the standards of the American Association of Blood Banks and the Federal Drug Administration issued a prohibition against the mixing of these solutions with anticoagulated blood products. This prohibition appears to be mostly based on the recommendations of 1975 Ryden and Oberman in vitro study. Lactated Ringer's solution contains 3 milliequivalents of calcium per liter and can theoretically activate the clotting cascade in stored packed red blood cells because of the previous chelation of the blood calcium with that of the citrate anticoagulant. Other in vivo studies have shown that

there are no adverse effects among patients when lactated Ringer's solution is used in situations when packed red cells are transfused rapidly, as in trauma or in a perioperative setting.

This research was a descriptive study that partially replicated the work of Ryden and Oberman (1975) using the methodology of Cull, Lally and Murphy (1991) in order to validate or refute Ryden and Oberman's (1975) recommendation that lactated Ringer's solution should never be administered concurrently with blood or that may be harmful when used to start transfusions as these mixtures rapidly produced clots. Cull et al. used the mixing of packed red blood cells with lactated Ringer's solution as the focus of their study. These authors discovered that no clots formed in the clinical relevant dilutions between 5:1 and 2:1 (packed red blood cells to lactated Ringer's solution). Their study also compared the flow rates of packed red blood cells diluted with lactated Ringer's solution to the flow rates of packed red blood cells diluted with normal saline. They found that there was no difference in flow rates between these solutions. Cull et al. recommended that 150 milliliters of lactated Ringer's solution can be safely used to dilute packed red blood cells in patients who require rapid blood transfusions.

The methodology of this thesis' research followed that of Cull et al. (1991) with the exception that the samples of diluted packed red blood cells were examined only macroscopically using orangewood sticks. The clots were also graded according to the scale provided by Strautz, Nelson & Schulman (1989). The results of the present study were remarkably similar to that of Cull et al. (1991). The slight differences in results were most likely due to minor technical differences. Data in both studies appeared to be very

reliable, as there were very little differences among the individual data points. The minor differences in the initial clotting times of the mixtures may be due to the completeness of the mixing of the samples. Certainly the techniques of this study were valid, as the results of the experiments within a given volume dilution were the same for all samples. Differences in the flow rates between the studies may have been due to the degree of mixing of the samples, the height at which the units of packed red cells were hung prior to flow, the length of the 16 gauge IV catheter, the brand of IV tubing, or the condition of the blood administration sets.

Even though it appears that under some circumstances that it would be possible to safely mix anticoagulated red blood cells with lactated Ringer's solution, it should not be a common practice. Even though the previously mentioned studies have presented the notion that there are no adverse effects on patients who received packed red cells diluted with lactated Ringer's solution, the potential exists that blood clots may be produced by mixing lactated Ringer's solution with anticoagulated packed red cells.

Prudence should dictate that when patients well being is considered, it is always best to err on the side of safety.

Further research should be conducted in the area of compatibility of anticoagulated packed red cells or other blood products with lactated Ringer's solution. The present study used packed red blood cells that were at the end of their shelf life, the study could be repeated using fresher packed red blood cells. The study could be expanded to include examination of the precise flow rates at which clots begin to form in the intravenous tubing.

The data presented in this study basically corroborates that of previous published reports. RL when added to PRBCs at some dilutions will produce small macroscopic clots. Unlike dextrose solutions, no hemolysis was observed. Small volumes of RL may appear to be compatible when added to PRBCs used for transfusion. This may explain in part the observations that patients who have received this admixture (RL + PRBCs) do not appear to suffer any obvious clinical complications. The data supports the observation that certain amounts of lactated Ringer's solution appear to be compatible with anticoagulated red blood cells.

The work of Cull et al. (1991) was corroborated since it was able to be replicated. The conclusions drawn by these authors appear to be valid. There is no difference in flow rates between packed red cells preserved with CPD and diluted with lactated Ringer's solution or normal saline. There were also no clots formed in clinically relevant volume dilutions of packed red cells with lactated Ringer's solution.

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APPENDIX

Sizing of Clots

0	no reaction
1+	<1mm
2+	1-2 mm
3+	2-4 mm
4+	>4mm or solid clot